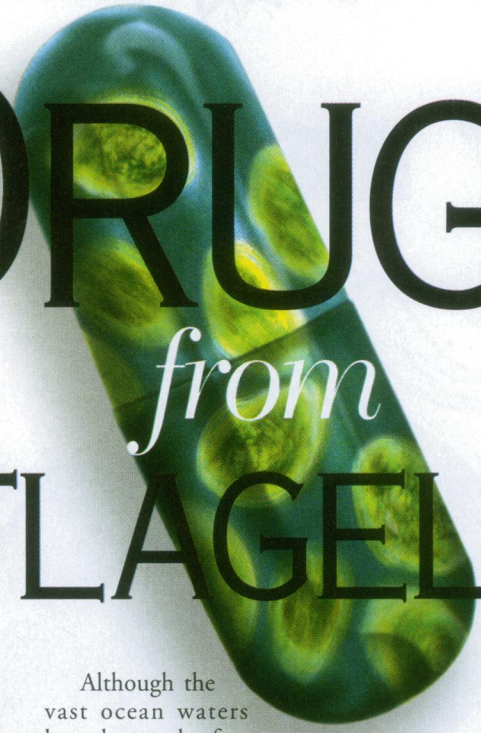
DRUGS *from* DINOFLAGELLATES



To fight both old and new diseases, scientists search continually for new pharmaceuticals. Many new drugs may soon come from compounds produced by marine animals and plants, and the great diversity of marine organisms could potentially lead to an equally diverse array of drugs. David Newman, a chemist in the natural products branch of the National Cancer Institute, explains the interest in marine compounds by considering a coral reef. "On a coral reef," he says, "the only way that an organism can survive is if it has a foothold, or toehold, because these [organisms] are almost all filter feeders. Therefore, if you have two sponges sitting alongside each other, you will occasionally see a demarcation line, almost as if they come up to each other and stop. Basically what you've got is chemical warfare going on there. You've got a quest for space, and as you go in and start looking you find some absolutely fascinating chemistry." That chemistry might produce equally fascinating pharmaceuticals.

Coral reef organisms alone may soon spawn a variety of new drugs. For example, the plant-like bryozoan *Bugula neritina* produces a compound called bryostatin 1, which is in Phase II clinical trials as an agent against melanoma, lymphoma, and nephroma. *Dolabella auricularia*, a type of nudibranch, or shell-less mollusk, makes dolastatin 10, an agent that looks promising against cancer. Beyond coral reefs, similar potential may exist in other marine organisms. In fact, thousands of compounds have been isolated from marine organisms in the past two decades.

Although the vast ocean waters host thousands of potentially powerful drugs, the organisms that make them can be so rare or inaccessible that scientists cannot collect and test the compounds. Says Yuzuru Shimizu, a natural-products chemist at the University of Rhode Island in Kingston, "A lot of compounds have been isolated from marine animals, and they are very interesting compounds. Many of them probably have the potential to become drugs. The drawback is that they are found only in very minute quantities. Also, the animals can be scarce and it is not easy to collect large quantities to isolate enough material to do some preliminary tests." Shimizu hopes to farm these organisms through large-scale aquaculture so that pharmaceutical companies can harvest as much of a compound as needed.

Drugs from Dinoflagellates

In 1970, Shimizu began studying bioactive metabolites of microalgae. He tested some of the compounds for their pharmaceutical potential by screening them against cancerous cell lines in tissue culture. Such screening tests sparked Shimizu's interest in dinoflagellates, or single-celled phytoplankton.

To determine what compound is produced by a specific dinoflagellate, Shimizu needed a culture that included only that organism. To prepare such a culture, Shimizu first collected organisms from the ocean. Then, using a micropipette, he hand-selected a single dinoflagellate under a microscope. That organism was cultured and allowed to multiply. As the organism's numbers increased, the culture was moved from a tiny well that held a few drops of seawater to a container the size of a test tube, and then to an even larger container. The culture was also treated to eliminate other microorganisms. The end result of the procedure was a culture composed of a single species, with no contaminants.

Once Shimizu had a pure culture, he looked for compounds. He took as many dinoflagellates as he could get from a culture, ground them up, and tried to extract individual compounds. Using various forms of chromatography, Shimizu separated the homogenized dinoflagellates into chemical samples. These samples were run through more chromatographic procedures and further purified in hopes of isolating a single compound. In collaboration with Shimizu, Bristol-Myers Squibb tested the compounds for their potential in fighting cancer. Unpublished results showed that one compound, carbenolide-I, shrank tumors when tested *in vivo* in mice with leukemia.

Isolating Amphidinolide

After a collecting trip near St. Thomas in the Caribbean, Shimizu started culturing a dinoflagellate of the genus *Amphidinium*. He and his colleagues worked for two years, growing batch after batch of *Amphidinium* in 150-liter tanks. Eventually, Shimizu had 6,000 l of culture. From that, he isolated 50 mg of a compound called amphidinolide B.

This compound stirred great interest.

In 1993, Jun'ichi Kobayashi and Masami Ishibashi, both pharmacologists at Hokkaido University in Japan, wrote in *Chemical Reviews*, "It should be noted that the cytotoxic activity of amphidinolide B and its related compounds are extremely strong." In particular, they reported that amphidinolide B killed cultures of a mouse leukemia cell line and a human carcinoma cell line. Consequently, Shimizu saw the potential of this compound, but he says that 50 mg was "not enough to do any further biological tests. So we had to get much larger quantities." He adds that there are 20–30 other interesting compounds produced by *Amphidinium*, but they are even scarcer in this dinoflagellate. For example, he obtained 10 mg of carbenolide-I, which he says is an anticarcinogen 100 times more potent than amphidinolide.

Cranking up the Culturing

After taking two years to make 6,000 l of *Amphidinium* culture, Shimizu knew that he needed to increase the scale of his culturing. To do that, he turned to his own backyard, the Marine Ecosystems Research Laboratory at the University of Rhode Island, which includes 14 culture tanks, each capable of holding 15,000 l.

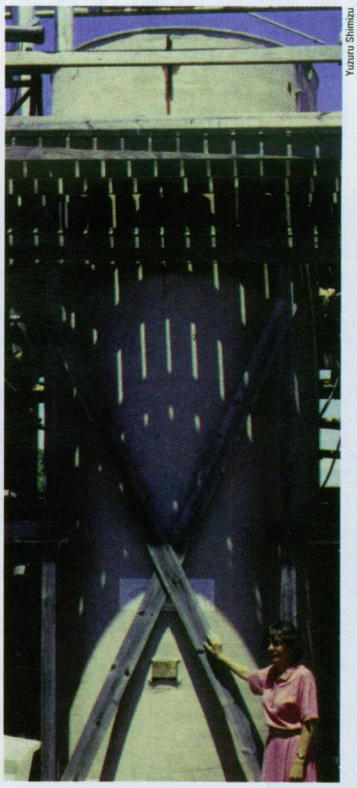
These cylindrical fiberglass tanks—5.5 m tall and 1.8 m in diameter—were originally designed for environmental studies. Consequently, they include computer controls for temperature, lighting, stirring, and other variables. Lucie Maranda, a marine research scientist at the University of Rhode Island's Graduate School of Oceanography, explains that it takes 15–20 carboys—each containing 12 l of *Amphidinium* culture—to inoculate one of the large tanks. Then, she says, it takes 6–8 weeks to go from inoculation to harvest, depending on the species being cultured.

Once a culture reaches maturity—essentially multiplying to a high density—the dinoflagellates are harvested. First, Shimizu and colleagues add alum, or potassium aluminum sulfate, to a tank to make the dinoflagellates group together and sink to the bottom. Then the upper liquid can be pumped off, leaving a slurry of organisms and alum at the bottom of the tank. This mixture goes into a device much like the one that separates cream from milk. In this case, though, the device separates the dinoflagellates from the slurry. The end product—dinoflagellates—can be used to isolate compounds.

Shimizu says, "The large culture [system] is a completely new game." In this new approach, even the chemistry of the organism changes; they make metabolites at different times in their development. So Shimizu and his colleagues must now analyze the metabolic products throughout *Amphidinium*'s development in these large cultures. Shimizu says, "We have shown that we can grow this organism in large quantities. That, we feel, is a great accomplishment. We opened a way that we can procure, if necessary, a large amount of compound."

Shimizu succeeded in combining large-scale culturing with many types of chromatography to isolate the pure compound. Jon Clardy, a professor of chemistry at Cornell University in Ithaca, New York, says, "It's hard enough to get mixtures of amphidinolides. And it's even harder to get one pure."

Nevertheless, Clardy says, "Yuzuru was able to purify one. That's what he does better than anyone else. A number of people had worked on the amphidinolides, but he was the one who pulled out a pure compound so that it could be studied." Using that purified amphidinolide B, Clardy and his colleagues determined its structure by X-ray diffraction. The structure includes an enormous ring composed of 25 carbon atoms and one oxygen atom. Clardy says that knowing a compound's structure allows a chemist to consider synthesizing or modifying it.


Yuzuru Shimizu

A lot of *Amphidinium*. Huge 15,000 liter culture tanks allow researchers enough dinoflagellate to isolate the minute amounts of cancer-fighting compounds they contain.

Starting a Synthesis

Pharmaceutical companies synthesize compounds so that they can be made artificially to ensure a constant supply. A compound may also need to be synthesized or modified because, Newman says, "It is very rare that a material directly isolated from a natural source is the drug in the end. In general, what you get is either a structure that you then modify or information that allows you to make something similar but slightly different." A chemist can make those modifications through synthesis, building the compound from scratch. David Myles, a synthetic organic chemist at the University of California at Los Angeles, and his colleagues may soon synthesize amphidinolide B.

Hugo Eng, a graduate student of Myles's, has been attacking this synthesis problem through a convergent synthetic strategy, trying to make amphidinolide B from relatively large starting pieces. Still, Eng must synthesize the starting pieces—essentially quarters of the total compound that are similar in size and complexity—atom by atom. So far, he has synthesized all four pieces. He has also coupled different pieces together. To complete the synthesis he must couple all four components and, finally, close the structure's large ring.

Myles believes that synthesis and aquaculture complement each other. He says, "All of the information in the database we've built up on amphidinolides in our total synthesis would, in some way, be applicable to semisynthesis; in other words, modification of a natural product."

Mike May

Suggested Reading

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